Acyclovir Bioavailability in Human Skin

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Clinical experience demonstrates that oral acyclovir (ACV) is superior to topical ACV in treating recurrent cutaneous herpes simplex virus type 1 (HSV-1) infections. Cutaneous HSV-1 infections are complex in their pathology, affecting the basal epidermis in skin as well as establishing a latency phase in sensory ganglia. In vitro and in vivo human skin model systems were used in the present study to quantitate ACV disposition and absorption in skin and blood following two routes of administration and to investigate whether bioavailability differences were the result of insufficient drug delivery. Physicochemical and physiologic parameters determined from these experiments were used to develop a mathematical model to predict ACV disposition and absorption in human subjects. Model predictions and in vivo data agree; topical administration of commercial 5% ACV ointment and cream result in a 48 times greater total epidermal ACV concentration than after oral administration. Mathematical modeling of the ACV concentration gradient through the epidermis revealed, however, that the drug concentration in the target site of HSV-1 infections, the basal epidermis, is 2–3 times less after topical administration than after oral administration. Thus, the observed lack of clinical efficacy with topical ACV therapy in the recurring HSV-1 infection likely reflects the insufficient delivery of the drug to the target site of the HSV-1 infection, the basal epidermis. *J Invest Dermatol 98:856–863, 1992*

The topical application of drugs for treatment of skin disease or pathology has a long history. There are an increasing number of reports, however, in which topical acyclovir therapy is not as effective as oral administration in the treatment of herpes simplex virus type I infections (HSV-1) [1–8]. Lack of efficacy with topical acyclovir has been hypothesized to reflect the inadequate delivery of drug to the skin [4,9,25]. The pathology and target site of the cutaneous disease and the mechanisms governing effective drug delivery to that site must necessarily be understood to design topical formulations that efficiently and effectively treat the disease. In the case of acyclovir (ACV), which is used for the treatment of herpes simplex virus-1 (HSV) infections, the target site of the HSV-1 lesion is the basal epidermis.

To understand why one route of administration might be superior to the other, the present investigation was designed to quantitatively assess ACV bioavailability in the epidermis and systemic blood following oral and topical ACV administration in vitro and in

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Abbreviations:

ACV: acyclovir
A_D: contact area between donor cell vehicle and skin (cm^2)
C^o_D: drug concentration entering the flow cell (mg/ml)
C_D^{for}: drug concentration in the commercial formulation (mg/ml)
D_m: diffusion coefficient for drug in skin (cm^2/h) (one-layer skin model)
D_{sc}: diffusion coefficient for drug in stratum corneum (cm^2/h)
D_{epi}: diffusion coefficient for drug in epidermis (cm^2/h)
EDTA: ethylenediaminetetraacetic acid
h: thickness of stratum corneum barrier (cm)
HPLC: high-pressure liquid chromatography
HSSF: human skin sandwich flap
HSV-1: herpes simplex virus type 1
IDI_{50}: 50% inhibition of viral cytopathic effect
IPM: isopropyl myristate
k_{12}: first-order rate constant for transfer of drug from body compartment number 1 to body compartment number 2 (h^{-1})
k_{21}: first-order rate constant for transfer of drug from body compartment number 2 to body compartment number 1 (h^{-1})
K_D: skin- static-cell vehicle partition coefficient at x = 0
k_{el}: first-order rate constant for elimination of drug from body compartment number 1 (h^{-1})
K_F: skin-flap blood partition coefficient at x = h
K_m^{eb}: epidermis-blood partition coefficient
K_m^{epi}: epidermis-vehicle partition coefficient
K_m^{sc}: stratum corneum-vehicle partition coefficient
K_m^{for}: stratum corneum-formulation partition coefficient
K_m^{app}: apparent heat-separated skin-vehicle partition coefficient
N: the number of nodes in the skin barrier
OHNM: orthotopically grafted human skin on nude mouse
PBS: phosphate-buffered saline, pH 7.2
PEG 400: polyethylene glycol molecular weight 400
PMSF: phenylmethyl-sulfonylfluoride
TS: tapestrip
v: volumetric blood flow rate between the rat flap and the central rat compartment or blood and dermis compartment (cm^3/h)
V_1: volume of distribution for rat (cm^3)
V_{derm}: volume of dermis compartment (cm^3)
V_D: static donor cell volume (cm^3)
V_F: rat flap volume (cm^3)
Vol_{epi}: tissue volume (cm^3) of the epidermis
Vol_{SC}: tissue volume (cm^3) of the stratum corneum
Vol_{epi+SC}: tissue volume (cm^3) of the epidermis + stratum corneum
vol: skin volume (cm^3)

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vivo. Assessment of ACV bioavailability in human skin in vivo was investigated with three human skin model systems: human subjects, human skin grafted orthotopically onto congenitally athymic mice (OHNM) [12], and in a rat/human skin sandwich flap on athymic rats (HSSF) [13]. The kinetics of drug delivery and elimination from the skin and into the systemic blood can be ascertained with the OHNM, whereas drug disposition and the flux of the topical drug across human skin can be directly quantified with the HSSF model.

A variety of in vitro model systems were utilized to gain an understanding of ACV absorption and transport through human skin. Physicochemical parameters measured in vitro, together with pharmacokinetic parameters quantified in vivo, were used in a mathematical model to further elucidate drug disposition in human skin and to predict in vivo ACV bioavailability. Model predictions and skin disposition of ACV in human subjects were subsequently compared.

MATERIALS AND METHODS

Chemicals Chemicals were used as purchased: acycloguanosine (Sigma, St. Louis, MO); [^{3}H] acyclovir (specific activity 10–25 μCi/mmole, New England Nuclear, Boston, MA); ethanol (denatured with 5% isopropyl alcohol, Aldrich Chemical Co., Inc., WI); and 5% Zovirax ointment (Burroughs Wellcome Co., Research Triangle Park, NC, purchased from the University Hospital Pharmacy). The 5% Zovirax modified aqueous cream was a gift from Burroughs Wellcome Co., Research Triangle Park, NC. Polyethylene glycol 400 (PEG 400), isopropyl myristate (IPM, 98%), trypsin (Type IX), ethylenediaminetetraacetic acid (EDTA), and phenylmethyl-sulfonylfluoride (PMSF) were purchased from Sigma.

Skin Samples Abdominal female human skin from elective abdominoplasty surgery was received within 4 h of surgery. Split-thickness skin samples (0.5 mm) were dermatomed and stored at 4°C for up to 2 weeks in tissue culture medium with the media changed every 3 d (Dulbecco's modified Eagle's media with 5% fetal calf serum, Flow Laboratories, McLean, VA). Permeability coefficients of ACV in vitro did not significantly change ($p > 0.05$) over this storage period.

Isolated stratum corneum and stratum corneum with intact epidermis were separated from split-thickness skin by trypsinization and heat separation, respectively, as previously described [14].

Analytical Techniques Nonradiolabeled ACV was detected and quantitated in all permeation experiments with reversed-phase (C-18 RP column: 5 μ; 4.6 mm × 25 cm, Beckman Ultrasphere ODS, San Ramon, CA) high-performance liquid chromatography (HPLC) (Beckman binary 110B Solvent Delivery Modules, 427 Programmable Integrator) at a fixed UV wavelength of 254 nm (Beckman programmable 160 Absorbance Detector, Palo Alto, CA) using a 0.5% acetic acid mobile phase (1.4 ml/min) and a 20-μl sample loop. Good separation of ACV (RT = 8 min) from other interfering peaks in the extracted skin and blood samples, as well as receiving chamber samples, were obtained with this method. Standard curves of ACV in 40/60 (v/v) PEG 400/water vehicle were linear from 0.05–5 μg/ml day to day, $r = 0.9984 \pm 0.0020$ (mean ± SD; n = 15). Limits of sensitivity were 0.05 μg/ml or 1 ng on column.

The radiolabeled drug was quantified in the experimental samples with the procedure described previously [14]. Thin-layer chromotographic analysis of [^{3}H] acyclovir revealed no significant impurities before or after experimentation (99% pure).

Generation and Extraction of Skin Samples Fresh biopsies collected from the various experiments were tape stripped to remove stratum corneum and then manually sectioned into two additional skin layers, epidermis and dermis, at room temperature with a technique using cyanoacrylate cement and a single-edged razor blade [15]. Extraction of the various skin layers was accomplished by adding 250–500 μl of 0.1 NaOH to the sample, vortexing on high speed for 30 seconds, and incubating the samples in a 90°C water bath for 5 min. Repeating the vortexing and 90°C incubation step insured a > 95% extraction efficiency. The alkaline extract was then submitted to HPLC analysis. The drug content in the various skin layers was determined by comparing the area-under-the-concentration curve with known acyclovir standards. Drug concentration in the individual skin layers was determined by dividing the total drug content in the skin layer by the volume (cm^3) of the tissue sample. The volume of tissue was calculated from the product of the surface area of the biopsy (cm^2) and the skin-layer thickness of the skin layer as dictated by the manual skin-sectioning technique (cm) and known biopsy thickness [15]. A typical 0.5-mm-thick biopsy was assumed to have a thickness of 40 μm for hydrated stratum corneum, 115 μm for the epidermis, and 460 μm for the dermis.

Topical and Oral Acyclovir Disposition in Human Skin

In Vitro: ACV distribution in split-thickness human skin (0.5 mm) was investigated in vitro using modified Franz cells, following topical application of commercial 5% acyclovir cream and ointment formulations (500 mg formulation/cm^2) into a 1.6-cm diameter plexiglass well for 0.5, 24, and 48 h. At the termination of the experiment, the residual drug was removed with a Teflon spatula and the skin surface gently wiped with three independent cotton applicators. Fourteen-millimeter diameter skin biopsies were subsequently collected from the treated split-thickness human skin with a cork borer. The biopsy was tape stripped 3–4 times with Transpore tape (3M, St. Paul, MN), then manually sectioned into epidermis and dermis skin layers, as described above. The tape strips were weighed before and 30 min after tape stripping on a Sartorius Ultramicro balance (model 4515 MP8 with a sensitivity of 0.1 μg) to quantify the stratum corneum weight removed.

Drug disposition of ACV in human skin was also studied following oral administration, which was simulated in vitro by adding tracer-radiolabeled drug to a 1 μg/ml solution of ACV in PBS pH 7.4 vehicle to the receiving chamber of a side-by-side diffusion chamber. The receiving chamber was sampled at the beginning and end of the 24-h experiment to document any change in the donor solution concentration of ACV. A 14-mm diameter biopsy was collected from the treated skin at 24 h following removal of the skin from the diffusion cell and blotted on tissues. The biopsy was tape stripped and manually sectioned into epidermis and dermis, as described above, and submitted to HPLC analysis.

In Vivo—OHNM: In vivo disposition of ACV in human skin following topical application of commercial 5% ACV formulations was quantitated using the orthotopically engrafted human skin on a nude mouse model (OHNM) [12]. All mice used for experimentation were healthy (no more than 20% weight loss from surgery), with intact human skin grafts, visually free of lesions with a functioning microcirculation, as assessed by laser Doppler velocimeter. Three to five weeks after engraftment, the resulting 1 cm^2 of human skin graft was treated with a single dose (14 mg formulation/cm^2) of commercial 5% ACV ointment or cream for 0.5 or 24 h. At the end of the dosing interval, the residual formulation was removed with three independent dry cotton applicators and the site was biopsied with a 3-mm diameter disposable punch biopsy (Accuderm, Ft. Lauderdale, FL). The biopsy was then tape stripped 11 times with Transpore tape (3M, St. Paul, MN) and sectioned manually into the epidermis and dermis, as previously described [15]. The resulting thicknesses associated with each skin layer of a typical 0.5-mm thick biopsy from the HSSF using this technique are assumed to be 40 μm for the fully hydrated stratum corneum, 115 μm for the epidermis, and 460 μm for the dermis [15]. The skin layers were placed into 1.5 ml polypropylene microcentrifuge tubes and stored at −70°C until extracted for HPLC drug analysis.

In vivo—Human Subjects: In vivo ACV disposition in human stratum corneum following unoccluded topical administration for 0.5 and 24 h was also performed in three human subjects, naive to ACV therapy, ranging in age from 19 to 38 years of age. Fourteen milligrams of commercial 5% ACV cream and ointment formulations was applied to the ventral forearm skin surface inside a 1-cm diame-

ter Teflon well attached to the skin surface with a dual adhesive disc (3M, St. Paul, MN). The treated skin area inside the Teflon well was covered with one layer of open loose-weave stretch conform gauze (Kendall, Mansfield, MA) to keep the treated area intact and protected from the environment over the dosing interval, without occlusion. Following the appropriate dosing interval, the residual formulation was lightly removed with a Teflon spatula and the drug-treated area gently wiped with three independent dry cotton swabs. The drug-treated skin site was then tape stripped 11 times with 0.6-cm diameter Transpore tape strips generated immediately before use with 0.6-cm diameter disposable punch biopsies (Accuderm, Ft. Lauderdale, FL). The first tape strip was discarded due to potential contamination of residual drug. The remaining 10 tape strips were combined into a 1.5 ml polypropylene microcentrifuge tube and stored at −70°C until extracted for HPLC analysis for drug quantitation.

MATHEMATICAL MODELING

Physicochemical Parameters

In Vitro: Solubility of ACV in various vehicles was measured by adding excess ACV to a vehicle volume of 1.5 ml in a 1.5-ml polypropylene microcentrifuge tube and vortexing the solution at high speed for 1 min. The solution was set aside at room temperature (25°C) with periodic mixing for over 24 h. The solutions were then recentrifuged for 15 min at 800 rpm. The supernatent was decanted, diluted, and analyzed for drug content with the HPLC method.

Partition coefficients of ACV from IPM: water were measured at 25°C by adding radiolabeled ACV (specific activity, 6×10^{-4} μCi/ml) to a 2-ml flint-glass vial containing 1 ml of both IPM and water, vortexing the vial vigorously, and allowing the two organic phases to separate over a 1-week time period. Radioactivity in a 200-μl sample collected from the IPM and water phases was determined by liquid scintillation counting (Packard 1900CA, Downers Grove, IL).

Partition coefficients of ACV between human stratum corneum and the vehicles of interest were performed with isolated desiccated stratum corneum [14] at 32°C for 1 week. Partition coefficient experiments were performed as previously described [14], with the exception that the skin samples were desiccated for 48 h and weighed on a Sartorius Ultra Micro Balance prior to placement in radioactive solution. Partition coefficients were calculated by first fitting a regression line through the partition coefficient data [14] in which the skin concentration, μg of drug/gm of desiccated skin, was the independent variable and the vehicle concentration, μg of drug/ml of solution, was the dependent variable. The resulting partition coefficient, based on the desiccated weight of the skin samples, was then converted to a volume-based partition coefficient by multiplying by the skin density. Skin density was estimated by dividing the average desiccated weight of the skin samples by the estimated volume of vehicle-equilibrated skin. This change in procedure allows for a more accurate measurement of the partition coefficient, accounting for the variability in the amount of skin volume used in each partition experiment.

Experiments performed to determine ACV partition coefficients between human stratum corneum + epidermis and water resulted in an alteration in the structural integrity of the epidermis from cellular to gel-like during the course of the experiment. Dissolution of epidermis in the presence of water was further examined by placing heat-separated skin in a 0.5 mg/ml ACV water solution containing protease inhibitor (0.1 mM PMSF and 1 mM EDTA). Solutions with and without protease inhibitor were incubated at 32°C for several days and dissolution of epidermis was monitored. The apparent epidermis-vehicle partition coefficient was estimated from

$$K_m^{epi} = \frac{vol_{(epi+SC)}}{vol_{epi}} K_m^{app} - \frac{vol_{SC}}{vol_{epi}} K_m^{SC}, \quad (1)$$

where "vol_{SC}" and "vol_{epi}" represent the vehicle-saturated volume of stratum corneum and epidermis, respectively, and K_m^{app} is the apparent partition coefficient of heat-separated skin when stratum corneum and epidermis are treated as one homogeneous layer.

Permeability coefficients of ACV across human skin in vitro were measured in symmetrical diffusion cells with vehicles containing water and 40/60 (v/v) PEG 400/water mixtures as described previously [14]. The skin samples were equilibrated with the water or PEG 400/water vehicles overnight prior to use in a permeation experiment to stabilize the skin characteristics during the initial permeation process.

Absorption of ACV into and across split-thickness human skin (300–400 μm) in vitro was also measured following topical application of commercial ointment and cream formulations (14 mg/cm²). Drug concentration in the skin was calculated as the amount of drug in a given volume of skin. The volume of skin was ascertained by using a specific surface area of a skin biopsy and assuming in these experiments the thickness of fully hydrated stratum corneum to be 40 μm, 80 μm for epidermis, and 200 μm for dermis. ACV skin absorption after systemic delivery was simulated in vitro by placing radioactive ACV in the receiving chamber of a Franz cell containing PBS solution. The dermis side of split-thickness skin was placed in contact with the drug solution for 48 h, after which the skin was tape stripped and then sectioned into epidermis and dermis [15] and analyzed for radioactivity by liquid scintillation counting (Packard model 1900CA, Morton Grove, IL).

In Vivo: In vivo ACV permeation experiments were conducted with the HSSF model. All rats used for experimentation were healthy (no more than 20% weight loss from surgery), with intact human skin grafts, visually free of lesions with a functioning microcirculation, as assessed by laser Doppler velocimeter.

^{3}H ACV in water (1.3 μg/ml) was applied to the human skin graft in an unstirred Teflon well (0.6 cm³) attached to the skin surface with a dual adhesive and sealed with Parafilm to prevent evaporation of the water vehicle. Blood collection (40 μl) from the vein immediately draining the flap (flap blood) and contralateral leg vein (systemic blood) were collected periodically from the rat over 4 h and analyzed for radioactivity using methods previously described [16].

Mathematical Model An unsteady-state mathematical model of in vitro ACV permeation [14] was used to best-fit diffusivity and skin thickness to the data obtained from side-by-side permeation experiments [14]. The in vitro permeation parameters, along with the volume of distribution obtained from the nude rat, were used in the development of a kinetic mathematical model of the HSSF [16]. This mathematical model was then used to predict the in vivo permeation of ACV across the HSSF. Another mathematical model was developed to predict drug concentrations in the stratum corneum, epidermis, and systemic blood after topical and oral administration of ACV in an oral-simulated clinical situation.

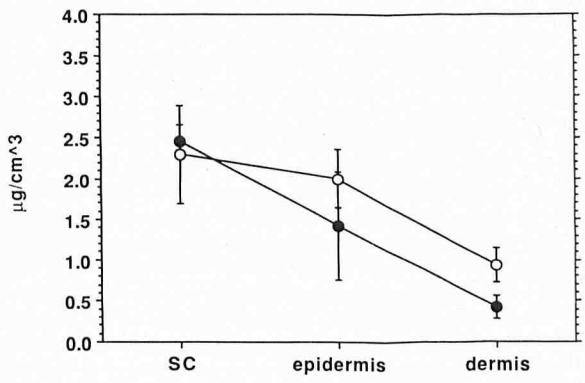

Figure 1. Acyclovir concentration in human stratum corneum (SC), epidermis, and dermis in vivo and in vitro after oral administration. Mean ± sem for n = 5. *Open circles,* in vitro; *closed circles,* orthotopic human skin grafts on nude mice.

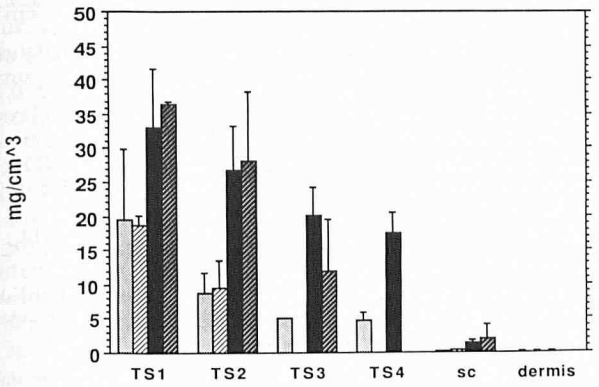

Figure 2. Uptake of acyclovir into split-thickness human skin in vitro following 24 and 48 h of 5% cream and 5% ointment. Mean ± SEM for n = 3. *Open stippled bar,* 5% cream for 24 h, *filled stippled bar,* 5% cream for 48 h. *Open hatched bar,* 5% ointment for 24 h; *solid hatched bar,* 5% ointment for 48 h.

Statistics Data between model systems were evaluated for statistical significance using the Student t test.

RESULTS AND DISCUSSION

ACV Disposition in Human Skin Following Oral and Topical Administration ACV disposition in the three human skin layers — stratum corneum, epidermis, and dermis — was evaluated in vivo using the OHNM after ACV gavage (3 mg/ml) and in vitro after introduction of 1 μg/ml of ACV into the receiving chamber of a modified Franz cell. Normalization of the systemic blood concentration of ACV in the OHNM model and the receiving chamber ACV concentrations to 1 μg/ml, the systemic blood concentration of ACV measured after therapeutic oral dosing [17], resulted in similar ACV concentration profiles in stratum corneum, epidermis, and dermis in vivo and in vitro (Fig 1). Stratum corneum ACV concentrations following oral-simulated administration in both in vitro and in vivo model systems were 1–2 times greater than epidermal and 2–6 times greater than dermal drug concentrations. The resulting concentration gradient (stratum corneum > epidermis > dermis) was the opposite of that expected following oral drug delivery based on diffusion from blood (dermis > epidermis > stratum corneum) and supported this drug's affinity for the stratum corneum.

Assuming equilibrium between skin and blood or PBS, the partition coefficient of ACV between the target site within the skin, the epidermis, and PBS (the vehicle) was about 2.

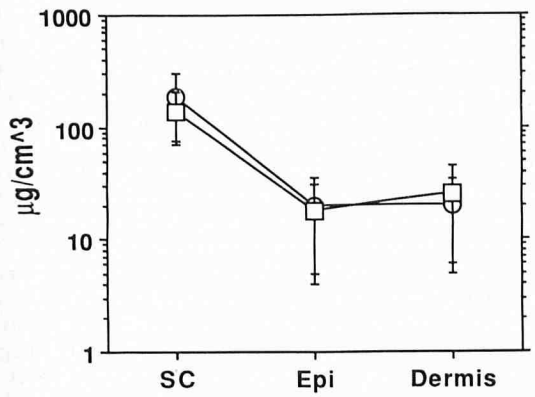

Figure 3. Acyclovir disposition in grafted human skin layers in vivo following topical administration of 5% cream and 5% ointment after 1 h. Mean ± SEM for n = 5. *Open circle,* 5% cream; *open square,* 5% ointment.

ACV Disposition in Human Skin Following Topical Administration In vitro cutaneous profiles of ACV in split-thickness (0.5 mm) human skin following topical drug administration of 5% cream and ointment for 24 and 48 h demonstrated concentration gradients through the skin, stratum corneum > epidermis > dermis at both dosing intervals (Fig 2). No significant differences in stratum corneum uptake of ACV into human skin were measured between the cream and ointment formulations at the same dosing interval. The drug concentration in all of the skin layers increased as a function of time; greater ACV concentrations are present in the stratum corneum and epidermal skin layers at 48 h compared with the same skin source at 24 h. This sharp increase in ACV concentrations observed after 48 h reflected the continual uptake of topical ACV over time in vitro and may be the result of ACV- or vehicle-induced changes in the stratum corneum in this model system.

Stratum corneum drug concentrations (corrected for volume of tissue) in tape strips 1 through 4 (TS 1 to TS 4) were 10–40 times greater than the remaining stratum corneum + epidermis after both dosing intervals. Epidermal ACV concentrations were twofold greater than dermis drug concentrations. Differences in the drug concentration in the skin layers were consistent with a concentration gradient through the skin. An approximate stratum corneum–formulation coefficient from these experiments was estimated to be 0.08 ± 0.04 at 24 h (mean ± SD, n = 8) and 0.23 ± 0.12 at 48 h in vitro, for both commercial 5% cream and 5% ointment.

Similar to the in vitro experiments, disposition of topical ACV in human skin in vivo in the OHNM revealed a concentration gradient of drug from the stratum corneum to the epidermis (Fig 3). One hour after topical application of 5% ACV cream or ointment, 7–10 times greater drug concentration was measured in the stratum corneum compared with the epidermis. The ointment formulation provided 20 ± 8 $\mu g/cm^3$ (mean ± SEM, n = 5) of ACV within the target layer of skin, the epidermis, where the HSV-1 infection resides. The modified 5% aqueous cream formulation provided 18 ± 7 $\mu g/cm^3$ of ACV to the epidermis. These tissue concentrations of ACV are 100 times greater than the 50% inhibition of viral cytopathic effect (IDI_{50}) required for HSV-1 infections, 0.35–0.79 μg/ml [18,19], and therefore suggest that topical ACV should be efficacious against HSV-1 infections. Whereas the clinical data for these topical formulations in the treatment of active recurrent herpes labialis supported the drug's influence in reducing viral shedding, they have failed to demonstrate any significant changes in the time to total healing, crusting or loss of pain [8], or frequency or severity of the recurrent lesions [20].

ACV uptake into the stratum corneum of split-thickness human skin after 0.5 h of topical 5% ACV ointment in vitro was 0.97 ± 0.18 mg/ml (mean ± SEM). This in vitro stratum corneum ACV concentration represented only one-half of the drug concentration measured in vivo after 0.5 h, in the OHNM and human subjects (1.95 ± 0.3 and 1.8 ± 0.4, respectively) (Fig 4). Increasing the in vitro dosing interval with the 5% ointment from 0.5 to 24 h resulted in ACV concentrations in the in vitro tape stripped stratum corneum (1.85 ± 0.38 mg/ml) that were non-significantly different ($p > 0.05$) from the in vivo data measured after 0.5 h. Thus, the kinetics of topical drug delivery to human skin in vitro were different from those in vivo. These data demonstrate the potential errors of using the same dosing interval in vitro to predict drug disposition in human stratum corneum in vivo. The mechanistic basis of this time-based discrepancy between drug uptake in vivo and in vitro is unknown, but likely reflects the greater hydration of the stratum corneum in vitro than in vivo. The similarity between ACV uptake into human stratum corneum in human subjects and human skin grafted onto nude mice, however, demonstrates the utility of the latter model system for evaluating the kinetics of topical drug delivery and elimination in human subjects.

Mathematical Modeling of ACV Disposition and Absorption Topical ACV administration in the present in vitro and in vivo experiments produced 100–1000 times greater epidermal concentrations than those documented concentrations (0.4–0.8

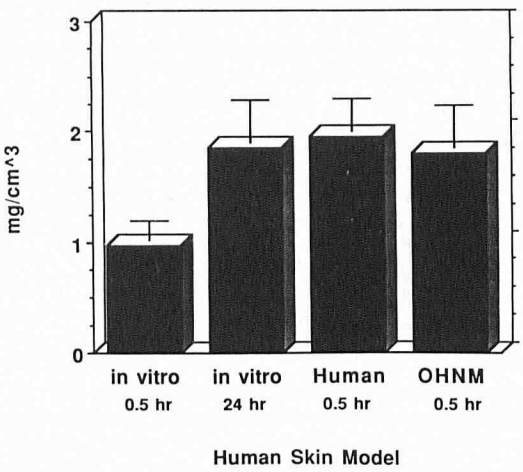

Figure 4. Comparison of acyclovir concentration in human stratum corneum in vitro and in vivo following a single topical dose of 5% acyclovir ointment. Mean ± SEM n = 5. *Human,* human subjects; *OHNM,* orthotopic grafted human skin on nude mice.

3.g/ml to 0.0214 μg/ml) required for a 50% inhibition of virus cytopathic effect [18–20], respectively). Further, topical administration of acyclovir in the present experiments produced tenfold greater concentrations in the total epidermis than oral administration of the drug. These data suggested that topical ACV treatment should be efficacious in treating cutaneous HSV-1 infections. These experimental data, however, are not consistent with clinical observations in which topical ACV administration generally failed to provide clinical efficacy in the treatment of HSV-1 infections.

Drug localization to the epidermis following topical and oral administration in the present experiments represented drug concentration in the entire epidermis, not the specific target site, the basal epidermis, where the HSV-1 infection resides. Quantitation of the basal epidermal ACV concentrations following oral and topical administration was difficult in the present experiments due to the small biopsy size and the manual skin sectioning technique employed. Drug concentration profiles through the epidermis could be obtained, however, using known physicochemical and pharmacokinetic parameters in a comprehensive mathematical model. This mathematical model was therefore utilized to explore the mechanisms involved in the clinical superiority of oral ACV administration above topical ACV administration for HSV-1 therapy.

A number of in vitro physicochemical parameters were required to adequately mathematically model the disposition and absorption of ACV in and across human skin in vitro and in vivo: solubility, partition coefficients between the vehicle and stratum corneum (as well as between the various skin layers), permeability and diffusion coefficients, and stratum corneum thickness.

Solubility The solubility of ACV in four vehicles — distilled water, PBS pH 7.4, 40/60 (v/v) PEG 400 : water, and 50% ethanol in water — was evaluated at room temperature (25 °C). ACV solubility in distilled water and PBS pH 7.4 were identical, 1.3 mg/ml. Adding PEG 400 to water in a 40/60 (v/v) mixture, respectively, increased ACV solubility twofold, to 2.6 mg/ml. Adding ethanol to water in 50/50 (v/v) mixture also increased ACV solubility twofold above water, to 2.8 mg/ml. Therefore, ACV solubility remained within the same order of magnitude, regardless of the vehicle hydrophilicity.

Permeation Parameters

Partition Coefficients: The estimation of ACV partitioning from an aqueous vehicle into skin was simulated in vitro with a water vehicle and IPM, which is commonly used to simulate the lipid within stratum corneum. The partitioning of ACV between water and IPM at 25 °C was measured with a drug solution consisting of a high specific activity (6×10^{-4} μCi/μl) in the water phase. Radioactivity in a 200-μl sample of the IPM phase was below background radiation levels. The IPM : water partition coefficient was estimated to be 1×10^{-5}. The stratum corneum : water partition coefficient for ACV was 0.5 ± 0.08 (mean ± sem, n = 36 from 4 skin sources) (Table I). Partition coefficients of ACV in isolated human stratum corneum differed significantly ($p < 0.05$) from the ACV partition coefficients determined with the skin lipid simulant IPM. These data indicated that successful mathematical modeling of ACV uptake into human skin would be best achieved using physicochemical parameters measured in the relevant tissue.

Table I. ACV Permeability Parameters in Human Skin

Vehicle	Water[a]	PEG 400/water[a]
Partition coefficient, stratum corneum	0.52 ± 0.03	0.49 ± 0.19
Partition coefficient, epidermis		1.22 ± 0.76
Diffusivity_{eff}[b] ($cm^2/h \times 10^{-7}$)	9.7 ± 8.5	ND[c]
$\text{Skin thickness}_{eff}$[d] (μm)	49 ± 43	ND[c]
$\text{Permeability coefficient}_{eff}$ ($cm/h \times 10^{-5}$)	10 ± 12	1.5 ± 0.9
Lag time (h)	5 ± 12	ND

[a] Mean ± SD (n = 36 for four skin sources).
[b] Effective diffusion coefficient describing the diffusion pathway through the skin as derived from the linear best-fit of the experimental permeation data to the complete unsteady-state mathematical model [14].
[c] Not detected.
[d] Effective skin thickness as calculated from the linear best -fit of the experimental permeation data to the complete unsteady-state mathematical model [14].

The slope of the regression line of drug concentration in the water vehicle against the drug concentration in a volume of skin had an average 5% standard deviation for both the stratum corneum and heat-separated skin. The drug did not bind to the skin, and therefore the linear regression was forced through the origin. Interestingly, when heat-separated skin (stratum corneum + epidermis) was used in these water-vehicle partitioning experiments, the epidermis was completely altered from a structural cellular integrity into a gel-like consistency. The epidermis : water partition coefficient could not, therefore, be accurately determined. The influence of the vehicle on drug partitioning into the stratum corneum and epidermis was investigated further with another vehicle, 40/60 (v/v) PEG 400/water. The ACV stratum corneum : 40/60 (v/v) PEG 400/water partition coefficient, 0.49 ± 0.19 (mean ± SD; n = 45, five skin sources), was not significantly different from that measured with the water vehicle. The epidermis of the heat-separated skin was not altered in the presence of the drug solution with this vehicle, and the epidermis : vehicle partition coefficient was therefore calculated from Eq. (1) (see *Materials and Methods*). The epidermis : 40/60 (v/v) PEG 400/water partition coefficient was 2.5 times greater than the stratum corneum : 40/60 (v/v) PEG 400/water partition coefficient of 1.22 ± 0.76 (mean ± SD; n = 36, four skin sources). These differential data of ACV partitioning into two skin layers indicated preferential drug uptake into the epidermis skin layer. The alteration of epidermal integrity in the presence of water vehicle has not been observed with other previously tested drugs (benzoic acid, estradiol, betamethasone dipropionate, progesterone, testosterone; data not shown) and thus highlights a potential detrimental property of high ACV concentrations in human skin.

The possibility that the observed alteration in epidermal integrity in vitro was a reflection of a drug effect on skin protease activity was investigated by comparing epidermal integrity of skin samples incubated at 25 °C in glass vials containing ACV + water + skin or water + skin, with or without protease inhibitor (EDTA + PMSF). The vials were examined periodically over a 2-week period for alteration in epidermal integrity. Greater epidermal dissolution was observed in the vials containing ACV than in those without ACV. The addition of protease inhibitor to the vials containing ACV inhibited alteration of the epidermis. No detrimental effect on the structural integrity of stratum corneum and dermis was observed histologically in any of the experiments. In both in vitro and in vivo permeation experiments where a concentration gradient of ACV existed across the human skin, the epidermal integrity was not sig-

nificantly altered. Therefore, the alteration of epidermal integrity n the partitioning experiments using heat-separated skin were ikely the result of high ACV concentrations in the epidermis, inducing epidermal protease activity.

Permeability Coefficients: Permeation of radiolabeled [^{3}H] ACV in water and 40/60 (v/v) PEG 400/water vehicles across human abdominal skin was measured in symmetrical side-by-side permeation cells [14]. The use of heat-separated skin was generally unproductive for analyzing the permeability resistance of ACV through the various skin layers because the heat-separated skin was susceptible to disintegration over the time of experimentation. In a selected number of experiments using heat-separated skin samples, however, permeabilities were measured similar to those measured across intact split-thickness skin from the same skin source. Thus, the primary resistance for the permeation of ACV across human skin resided in the stratum corneum, with negligible permeation resistance in the epidermis and dermis.

Permeability coefficients from in vitro permeation experiments using the water vehicle were calculated from the partition coefficient, effective diffusion coefficient, and effective skin thickness. The in vitro unsteady-state mathematical model was used to best fit the effective diffusivity and skin thickness to the permeation data [14], using the average total accumulated amounts of drug in the receiving chamber over 48 h (Table I). The absorption of ^{3}H ACV in the receiving chamber of the in vitro permeation experiments using the 40/60 (v/v) PEG 400/water vehicle was sufficiently low that the effective diffusion coefficient and effective skin thickness could not be reliably fit by the mathematical model. These data were consistent with the lack of systemic absorption (<0.1 μmol/l) measured clinically following topical 5% ACV in the polyethylene glycol formulation in vivo [20]. Permeabilities from experiments using the 40/60 (v/v) PEG 400/water vehicle were estimated from the slope of the apparent steady-state accumulation of ACV in the receiving chamber. Average permeabilities of ACV across human skin in vitro from the water and 40/60 (v/v) PEG 400/water vehicles were $1.0 \pm 1.2 \times 10^{-4}$ cm/h (mean ± SD, n = four skin sources) and $1.5 \pm 0.9 \times 10^{-5}$ cm/h, respectively. A sixfold greater permeability of ACV across human skin was measured in the water vehicle compared with the 40/60 (v/v) PEG 400/water vehicle, despite similar ACV partition coefficients in human stratum corneum and the greater hydration of the stratum corneum thickness by the water vehicle. The six-fold difference in permeability with the 40/60 (v/v) PEG 400/water vehicle likely reflects the lowered effective ACV diffusivity through the stratum corneum. Subsequent in vivo modeling utilized the diffusivity of ACV in the water vehicle, because in vivo skin is naturally perfused with blood, an aqueous medium.

A lag time of topical ACV absorption from a water vehicle across split-thickness human skin in vitro was 5 ± 12 h (mean ± SD for 36 samples from four skin sources) as determined from the linear best-fit of the accumulated amount of ACV in the receiving chamber over time. This lag time (5 h) was considerably longer than that lag time observed in vivo (5 min) and likely reflects differences in effective diffusion pathlength, state of hydration, and sink conditions between the two model systems. Absorption of ACV across human skin was negligible from the 40/60 (v/v) PEG 400/water vehicle and thus lag time was not determined.

Table II. Parameters for Modeling Permeation of Topical ACV in a Water Vehicle across the Human Skin Sandwich Flap In Vivo

Parameter or Constant	Value
A_D	0.785 cm^2
C_D^o	1.2 μg/ml
D_m	1×10^{-6} cm^2/h
h	0.0016 and 0.0020 cm
k_{12}	5.01 h^{-1}
k_{21}	3.98 h^{-1}
k_{el}	3.42 h^{-1}
K_F	2
K_D	0.5
N	21
v	30 ml/h
V_1	49.6 ml
V_D	1.0 ml
V_F	0.736 ml

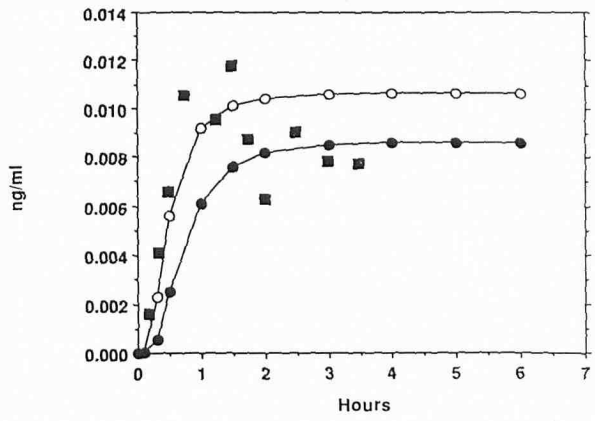

Figure 5. Comparison of experimental and model predictions of acyclovir permeation across the HSSF following 1.2 μg/ml solution of acyclovir in water. *HSSF,* human skin sandwich flap. *Solid squares,* acyclovir concentrations measured in flap blood of HSSF experiments. *Open* and *closed circles,* predicted flap blood concentrations using 16 μm and 20 μm stratum corneum thickness, respectively.

Topical ACV Absorption across HSSF Using a numerical model [16], the flap and systemic blood concentrations were estimated with values for the pertinent permeation parameters listed in Table II. ACV concentrations measured in the flap blood from HSSF experiments (closed squares) are plotted in Fig 5 along with the theoretically generated concentrations (open and closed circles with lines) over time. Radioactivity in the systemic blood samples was at or below the detection limits of about 0.003 ng/ml and therefore could not be compared with the estimated values generated by the theoretical model. Percutaneous absorption of 1.3 mg/ml ACV in a water vehicle across grafted human skin produced an in vivo lag time of less than 55 min. Maximal flap blood concentrations of 10–12 pg/ml were achieved at ~1 h, and thereafter decreased to 7.5 pg/ml. A major discrepancy was noted between in vitro and in vivo permeability coefficients when the HSSF permeation experiment was modeled. To obtain flap blood concentrations similar to the experimental data, the in vivo mathematical model required an ACV permeability roughly twice that measured in vitro. Effective permeability in the mathematical model could be increased by either increasing the diffusivity or decreasing the skin thickness. In vivo skin thickness was not expected to be as fully hydrated as in vitro skin, and thus, the barrier thickness used in model predictions was adjusted from the fully hydrated in vitro value of 49 μm to 16–20 μm, a value more consistent with the thickness of nonhydrated stratum corneum.

Using an in vitro skin thickness of 20 μm in the theoretical model, the predicted steady state flap concentrations (open square) were of the appropriate magnitude (Fig 5), but did not identify the rapid increase in flap blood drug concentration measured in the HSSF experiments (closed square) during the first hour. With a skin thickness of 16 μm (open triangle), the rapid increase in flap concentrations at early times post drug application were predicted well by the theoretical model, yet the steady-state flap blood concentrations were overestimated. The slowly decreasing flap blood concentrations of topical ACV in the in vivo HSSF experiments from 1–3 h may have reflected a decrease in the concentration of drug in the donor well, or an increase of blood flow through the flap at these time points, or an increase in barrier thickness resulting from progressive hydration. Neither the ACV concentration in the donor well, upon repeated sampling, nor blood flow through the flap, as

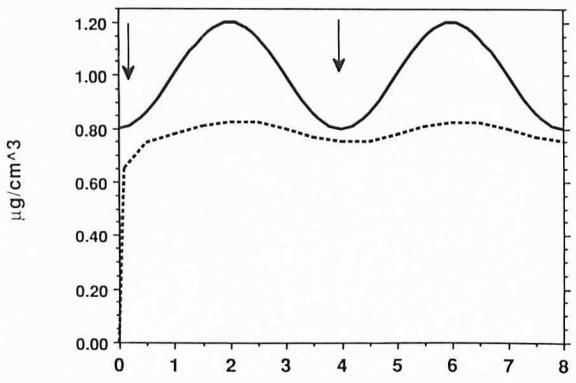

Figure 6. Model predictions of systemic blood and epidermal concentrations of acyclovir following oral acyclovir administration. *Solid line,* systemic blood; *dashed line,* epidermis; *arrows,* time of dose (every 4 h).

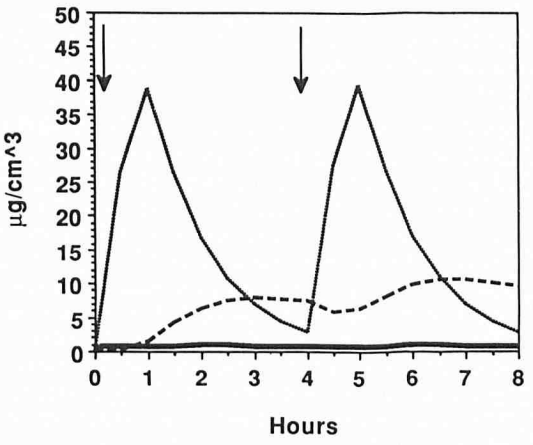

Figure 7. Model predictions of total epidermal acyclovir concentrations following oral and topical acyclovir administration. *Dotted line,* topical acyclovir administration using a 16-μm stratum corneum thickness; *dashed line,* topical acyclovir administration using a 40-μm stratum corneum thickness; *solid line,* oral acyclovir administration; *arrows,* time of dose (every 4 h).

monitored by the laser Doppler velocimeter, changed significantly over the time period of experimentation. Thus, the decrease in flap ACV concentration over time most likely reflects the progressive hydration and thickening of the stratum corneum over time. Comparison of mathematical model predictions based on in vitro physicochemical parameters with the in vivo data demonstrated, more importantly, that the effective diffusivity obtained from in vitro experiments is independent of the degree of skin hydration and can be used to predict in vivo permeation despite the differences in skin thickness.

ACV Disposition in Human Skin and Systemic Blood To better understand ACV bioavailability after oral and topical administration, theoretical skin concentrations of ACV were calculated with a two-layer mathematical model of skin. The main assumptions used in the mathematical model include: 1) an epidermis diffusivity three orders of magnitude higher than that of the stratum corneum; 2) an epidermis thickness of 80 μm; 3) application of topical formulation every 4 h; 4) a consistent contact of applied formulation with the skin surface at infinite source conditions during the first half-hour of each dosing interval; and 5) a sine function of systemic blood concentrations resulting from the absorption and elimination of the therapeutic oral administration of drug.

Total epidermal (dashed line) and systemic blood (solid line) concentrations of ACV after oral administration (Fig 6) were predicted with the parameters in Table III. Peak systemic blood concentrations of 1.2 μg/ml were predicted to occur 2 h after the oral dose and to be approximately 1.5 times greater than the peak total epidermal concentrations. Epidermal concentrations of ACV were relatively stable over time, despite the peaks and troughs of drug concentration in the systemic blood. An epidermis-to-blood partition coefficient of 2 was calculated from these data. These data indicated a preferred disposition of orally administered ACV to the epidermis. Model predictions agreed with the epidermal and systemic blood concentrations measured in vivo in the OHNM human skin model following oral administration.

Table III. Parameters for Modeling Permeation of Commercial Topical 5% ACV Formulations across Human Skin In Vivo

Parameter or Constant	Value
A_D	0.785 cm^2
C_D^{for}	50000 μg/ml
D_{SC}	9.7×10^{-7} cm^2/h
D_{epi}	9.7×10^{-4} cm^2/h
h	0.0016, 0.004 cm
k_{12}	1.14 h^{-1}
k_{21}	0.713 h^{-1}
k_{el}	0.92 h^{-1}
K_m^{eb}	2
K_m^{epi}	1.2
K_m^{for}	0.1
K_m^{sc}	0.5
N	31
v	0.0936 ml/h
V_1	20000 ml
V_{derm}	0.039 ml

Total epidermal concentrations of ACV following topical application at 0 and 4 h were a strong function of stratum corneum thickness (Fig 7). A gradual increase in epidermal ACV concentration was predicted for a hydrated stratum corneum thickness of 40 μm (dashed line), with maximal concentrations of 7.5 μg/ml occurring 3 h after dosing, thereafter decreasing to a trough concentration of 5.5 μg/cm^3 immediately before the next dose at 4 h. In contrast, the mathematical model predicted that topical ACV applied to a nonhydrated stratum corneum with a thickness of ~20 μm (dotted line) will produce sharp peaks and troughs of epidermal ACV concentration, with maximal concentrations of 38 μg/ml occurring at 1 h after dosing, thereafter decreasing 15 times to ~2.5 μg/cm^3 at 4 h. This 15 times difference in projected total epidermal drug concentration following topical administration to a 20-μm stratum corneum thickness was in contrast to the more stable drug concentrations predicted following oral administration.

Whereas the theoretical model projected peak and trough systemic ACV blood concentrations following oral administration of ACV (Fig 6), topical administration of the drug produced systemic drug concentrations less than 1×10^{-5} μg/ml per cm^2 area or analytically nondetectable. In vivo experiments using the OHNM confirmed the lack of detectable systemic ACV blood concentrations following the topical dose, using the limits of sensitivity in the current analytical method. Comparison of the theoretical and experimental epidermal ACV concentrations following oral and topical administration clearly demonstrates that topical administration of ACV produced significantly greater mean total epidermal drug concentrations than oral administration. The lack of ACV uptake into human skin from the topical vehicle formulation into skin was therefore not the mechanistic basis for the lack of clinical efficacy observed with topical ACV therapy.

HSV-1 infection and replication occurs in the basal epidermis. The concentration of ACV in this basal epidermal layer may therefore be critical to inhibiting viral replication. The concentration of ACV at the basal epidermis following both routes of administration could be quite different due to the direction of the drug concentration gradient, e.g., from the stratum corneum to dermis following topical and dermis to stratum corneum following oral administration. The theoretical prediction for ACV concentration in the basal epidermis based on experimental data collected following oral and topical administration (Fig 8) demonstrated that ACV concentra-

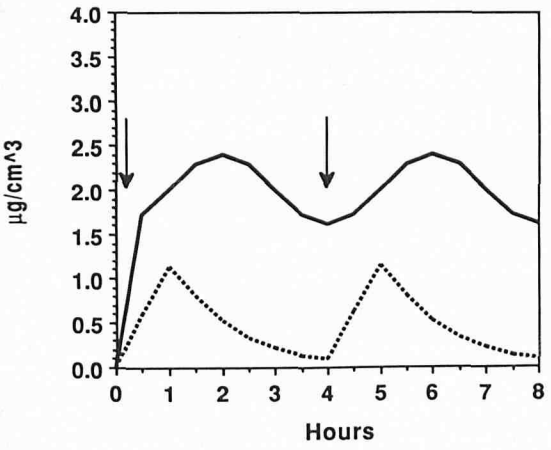

Figure 8. Model predictions of basal epidermal acyclovir concentration following oral and topical acyclovir administration. *Solid line,* oral administration; *dashed line,* topical administration; *arrows,* time of dose (every 4 h).

tion in the basal epidermis over the 4-h dosing interval was greater following oral administration than with topical administration. As expected with kinetics of oral dosing, the epidermal drug concentration maximized later than the systemic blood. Basal epidermal concentrations were predicted to decrease 12 times over the 4-h dosing interval following topical administration, whereas it decreased only 1.5 times following oral dosing. Thus, when the specific target site within the skin was considered, ACV concentration in the basal epidermis following oral administration was superior over time.

In summary, the route of ACV administration greatly influenced the delivery of the drug to the target site of the HSV-1 infection in human skin, the basal epidermis. Topical and oral ACV administration produced tissue drug concentrations in all three human skin layers in both in vivo and in vitro model systems. Whereas topical ACV administration delivered more drug to the entire epidermis, oral administration delivered more drug to the basal epidermis, where the infections resides. The delivery of greater amounts of ACV to the target site should also provide better therapeutic efficacy. These data and model projections provide a mechanistic rationale for the superior clinical treatment of HSV-1 infections by the oral route of administration [1–8]. Further, this study demonstrates how in vivo and in vitro experimentation in conjunction with mathematical modeling may provide further insight into the drug targeting of topical and oral drug therapy to the skin.

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